Scanning Electron Microscope Studies of Pulmonate Spermatozoa

BY

W. L. MAXWELL

Zoology Department, University of Bristol, U. K.

(3 Plates)

INTRODUCTION

The scanning electron miscroscope (SEM) is a useful tool for gaining a rapid appreciation of the gross form of single cells, such as spermatozoa, and a technique for the routine preparation of such cells had been described by BACCETTI & BURRINI (1973). However, their observations were confined to mammalian material. There has been no previous attempt to apply this technique to the much more complex spermatozoa of the pulmonate molluscs where an understanding of the gamete form has only been obtained from thin sections or gold shadowed preparations, as for example in *Agriolimax reticulatus* (BAYNE, 1970) or by means of the freeze-etching technique (THOMPSON, 1971, 1973). This lack of information may have contributed to the view that all pulmonate mollusc spermatozoa are practically identical (BAYNE, *op. cit.*). The SEM provides a tool for obtaining a rapid appreciation of the variety of form of the spermatozoa of the pulmonate molluscs.

METHODS

A direct application of the technique utilised by BACCETTI & BURRINI (1973) provided some preservation of the gross features of the cells but often resulted in extensive damage in the head region with the result that the sperm nucleus was mis-shapen or even exploded. A slight alteration in the constituents of the fixative was found to result in better preservation of the head form to provide information comparable with that obtained from freeze-etched replicas. The fixative was made up as follows: 1% potassium permanganate + 1% osmium tetroxide + 0.85% sodium chloride in distilled water. The pH was adjusted to 7.2 with NaOH.

Number 0 Chance coverslips were trimmed to cover but not overlap the edge of a standard SEM stub and after cleaning in absolute alcohol these coverslips were glued to the surface of the stub with electron-conductor glue. Fresh material obtained directly from the seminal vesicles of mature animals was smeared on the coverslips and a drop of fixative applied. The fixative was washed off after 20 minutes and the material washed for an hour before immersion in dimethyl sulphoxide for 30 minutes. The specimens were rapidly frozen in liquid Freon for 30 seconds before being freeze-dried and coated. Examination of the specimen was performed with a Cambridge Stereoscan S4 SEM in the Botany/Zoology E. M. Laboratories at the University of Bristol.

RESULTS

The investigation was carried out using mature spermatozoa from five species of pulmonate molluscs. The form of the spermatozoa of each species will be described separately. The only aspect that pulmonate spermatozoa seem to share is a tendency towards a helical organisation but the number of mitochondrial helices varies both between species and along the length of the sperm tail. This variation in helical form is also reflected in the form of the sperm head. The functional significance of these differences remains obscure.

Arion hortensis Férussac, 1819

The mature spermatozoon of *Arion hortensis* is 300 ± 5 μm in length. The sperm head forms one turn of a triple helix which is continued smoothly into the triple helix of the mitochondrial derivative (Figure *1*). Just behind the head the mitochondrial helices form marked keeled structures with a pitch of 4 ± 0.2 μm. The 3 helices gradually diminish in radial height and become more closely apposed (Figure *2*) towards the tail tip. At a point 72 ± 0.2 μm behind the head 2 of the helices are lost and a single helix with a pitch of 4.5 ± 0.2 μm continues along the remaining length of the spermatozoon. This single helix is large in proportion to the cross-section of the flagellum and is flattened around it with the result that the flagellum appears smooth in the posterior regions (Figure *2*, arrow).

Agriolimax reticulatus (Müller, 1774)

Bayne (1970) gives a mature spermatozoan length of 140 ± 5 μm and this was confirmed. *Agriolimax* differs from the other species here described in the complex organisation of the spermatozoan head. The head is 8 ± 0.2 μm long and has the form of a triple corkscrew which undergoes 2 revolutions in the length of the organelle (Figure *3*). However, the 3 ridges of the spiral do not run parallel since one is straightened anteriorly and carries the acrosome (Figure *4*) while the other 2 helices spiral around this 'core' (Figures *3* and *4*). The trihelical structure of the head is continued in the anterior portion of the mitochondrial derivative where the helices have a pitch of 4.0 ± 0.2 μm but it should be noted that even at a point immediately behind the head there is a disparity in the relative sizes of the helices where the so-called major helix is larger than the other 2 (Figure *5*). More posteriorly the 3 helices become flattened around the flagellum and the size disparity is further accentuated (Figure *6*). Near the tail tip the helical organisation of the mitochondrial derivative is lost altogether and a smooth tubular form is assumed (Figure *6*).

Milax sowerbyi (Férussac, 1823)

The mature spermatozoon of *Milax sowerbyi* is 246 ± 5 μm in length. The head describes 1½ gyres of 5 parallel helices and is surmounted by the helical acrosome. The whole head is 11 ± 0.2 μm long. The 5 helices are continued into the mitochondrial derivative but this differs from those of *Arion* and *Agriolimax* in the small size of these helices with the result that they are indistinct in longitudinal view (Figure *7*). However, it is evident that one helix is larger than the other 4 and this difference demonstrates a helical pitch of 5 ± 0.2 μm. The 4 smaller helices are lost about 31 ± 0.5 μm behind the sperm head and only the larger helix continues along the spermato-

Explanation of Figures *1* to *3*

Figure *1*: *Arion hortensis*: Sperm head and part of the flagellum showing the marked mitochondrial helices

Figure *2*: *Arion hortensis*: Part of the trihelical portion of the flagellum and the smooth posterior portions (arrow)

Figure *3*: *Agriolimax reticulatus*: Longitudinal view of the mature sperm head

Explanation of Figures *4* to *7*

Figure *4*: *Agriolimax reticulatus*: The apical acrosome surmounting one gyre of the helical nucleus

Figure *5*: *Agriolimax reticulatus*: Part of the anterior portion of the sperm tail showing the disparity in size of the mitochondrial helices

Figure *6*: *Agriolimax reticulatus*: Part of the mid-tail showing the large major helix and the smooth posterior region of the flagellum

Figure *7*: *Milax sowerbyi*: Part of the anterior portion of the sperm tail showing the small, indistinct mitochondrial helices

Explanation of Figures *8* to *11*

Figure *8*: *Lymnea peregra*: The head and anterior portion of a mature spermatozoon

Figure *9*: *Lymnea peregra*: Part of the anterior portion of the sperm tail showing slight disparity in size of the major helices (arrows)

Figure *10*: *Lymnea peregra*: Part of the mid-portion and posterior regions of mature spermatozoa showing the double helix in the mid-portion and the reduction in diameter of the posterior portion

Figure *11*: *Helix aspersa*: The head and anterior portion of a mature sperm showing the nearly smooth nucleus and the single, flattened mitochondrial helix

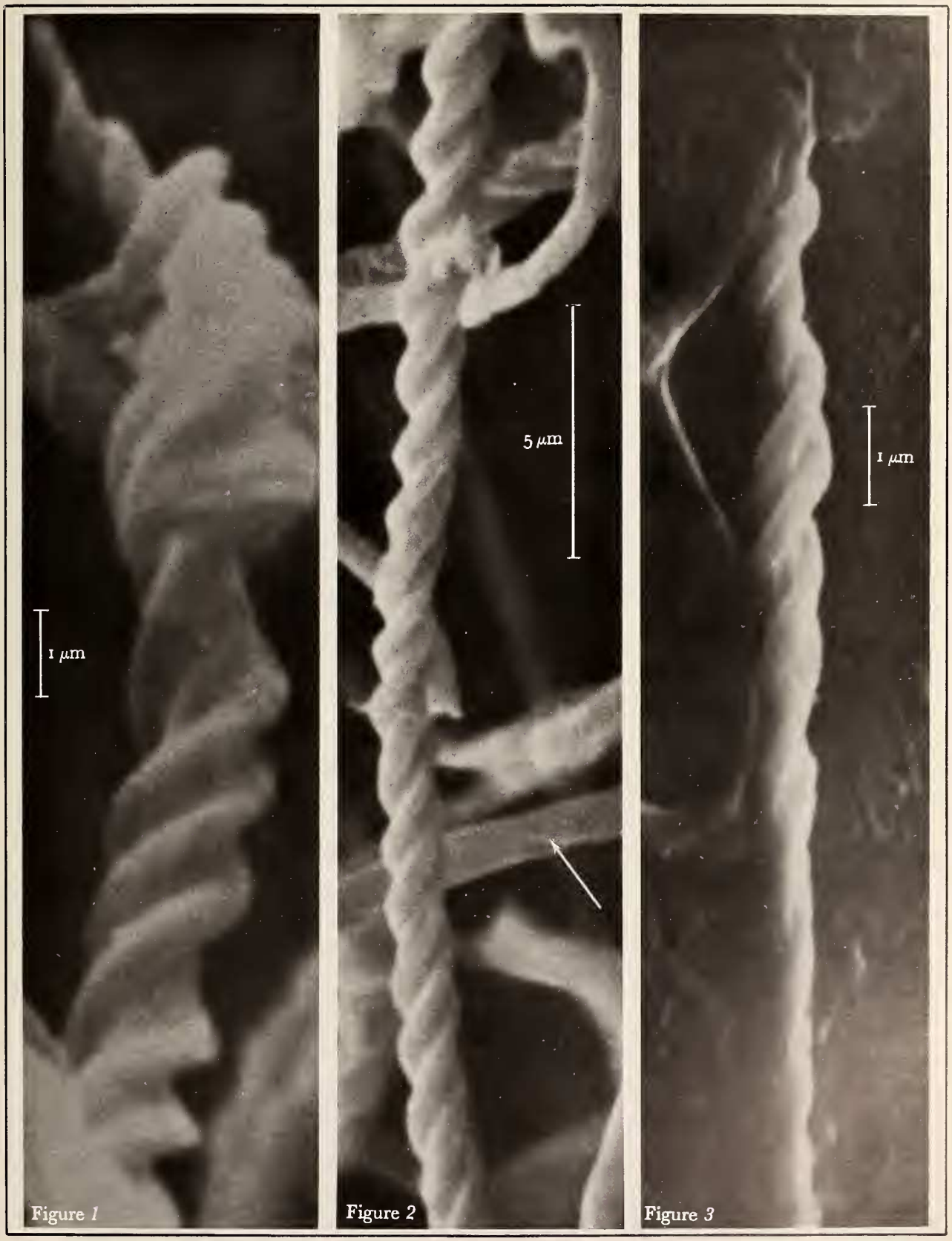

Figure *1*

Figure *2*

Figure *3*